Attachment I: Draft Approach to Exempting Certain PVCP-PIPs from Regulation under FIFRA

I. What Action Does this Paper Discuss?

EPA is considering whether to establish an exemption under FIFRA for certain plant-incorporated protectants that are based on one or more genes, or segments of genes, that encode a coat protein of a virus that naturally infects plants. Such plant virus coat protein plant-incorporated protectants are hereafter referred to as "PVCP-PIPs."

In accordance with EPA's regulations at 40 C.F.R. § 174.21, three general qualifications must be met for any PIP to be exempt from the requirements of FIFRA: (1) the PIP must meet a set of criteria specific to the particular PIP under consideration, (2) the residues of a PVCP-PIP that is intended to be produced and used in a crop used as food must either be exempted from the requirement of a tolerance under FFDCA or no tolerance would otherwise be required for the PVCP-PIP, and (3) any inert ingredient contained in the PIP must be codified at subpart X of 40 CFR Part 174 – List of Approved Inert Ingredients. The Agency is considering three criteria that would allow a PVCP-PIP to satisfy the first of these general requirements (i.e., the 174.21(a) requirement). Thus, the three criteria relevant to the 174.21(a) requirement are intended to address three issues that are associated with potential risks of PVCP-PIPs: gene flow (criterion (a)), recombination (criterion (b)), and protein production (criterion (c)). The criteria under consideration referred to throughout this document appear together in the Appendix of this attachment.

The PVCP-PIP would have to meet all three of the criteria in order to meet the 174.21(a) exemption requirement. A PVCP-PIP may be determined to meet each criterion in one of two ways: a product developer may self-determine that paragraph (1) of the criterion is met or the Agency may determine that paragraph (2) of the criterion is met. Paragraph (1) of each criterion describes an objective, well-defined characteristic. Therefore, the developer may determine whether the PVCP-PIP meets the requirement. Paragraph (2) of each criterion would be conditioned on an Agency determination because it may involve analysis of several types of information. Therefore, an Agency review would be necessary to evaluate and conclude that the PVCP-PIP meets the requirement and is therefore of a nature warranting exemption.

Each criterion may be satisfied under either paragraph (1) or paragraph (2) irrespective of how the other two criteria are satisfied. Thus, there would be no requirement that all three criteria must be satisfied under either paragraph (1) or paragraph (2) in order to qualify for the exemption.

Products that fail to meet one or more of the exemption criteria would need to obtain a registration. EPA would evaluate such PVCP-PIPs under the existing registration process and could implement control measures on use as appropriate.

If a PVCP-PIP does not satisfy a particular criterion under paragraph (1), EPA envisions that in order for a product to qualify for an exemption, the product developer would make a

submission to the Agency containing supporting data or other information to demonstrate that a particular PVCP-PIP meets paragraph (2) of that criterion to enable Agency review for that criterion.

II. Key Scientific Issues

Several scientific questions concerning risk issues associated with PVCP-PIPs have been identified: (1) What is the potential for a PVCP-PIP to endow plants with characteristics that could disrupt the existing network of ecological relationships in managed, semi-managed, or natural ecosystems, e.g., through gene transfer to wild or weedy[1] relatives?; (2) what is the potential for viral interactions to affect the epidemiology or pathogenicity of plant viruses?; and (3) what is the potential for exposure of humans or nontarget organisms to PVC-proteins with novel toxic or allergenic properties?

A. Potential for a PVCP-PIP to disrupt ecological relationships

In evaluating whether a PVCP-PIP could alter ecological relationships among plants, EPA considered two primary issues: (1) whether the PVCP-PIP could endow a transgenic plant itself with the ability to spread into natural or semi-managed habitats and (2) whether the transfer of a PVCP-PIP from a transgenic plant into wild or weedy relatives could disrupt ecological relationships. Whether gene transfer could disrupt ecological relationships depends on several additional considerations. First, does the crop plant containing the PVCP-PIP have wild relatives growing in its vicinity with which it is able to hybridize? Second, is virus infection limiting the growth and/or reproduction of individual plants within populations of wild or weedy relatives such that a gene conferring virus resistance is likely to become a stable part of the genome? Third, would stable introduction of a PVCP-PIP into the plant population (i.e., introgression) cause it to become weedier/more invasive or lose its competitive ability, thereby changing the population dynamics of the plant community?

1. Likelihood that a crop plant containing a PVCP-PIP could itself disrupt ecological relationships

In considering whether a PVCP-PIP could affect the ability of a plant to spread into natural or semi-managed habitat at the margins of cultivated fields, i.e., to form feral or naturalized populations, the key consideration is whether viral infection is currently limiting the ability of agricultural crops to do so. EPA is aware of no evidence suggesting that such is the case. For

[1] Throughout this document, EPA considers weedy plants to be those with the characteristics of weeds, i.e., those that are considered undesirable, unattractive, or troublesome, especially when growing where they are not wanted. Wild plants are those that occur, grow, and live in a natural state and are not domesticated, cultivated, or tamed. EPA considers a naturalized population to be a population of domesticated plants that grows in wild (non-cultivated areas). EPA considers a native plant population to be one that originates in a particular region or ecosystem.

example, field experiments with transgenic virus resistant sugar beets revealed no competitive advantage (measured as seedling emergence and biomass production) between the transgenic and susceptible control lines (Ref. 1). There are also no reports that conventional control of virus pathogens, e.g., by virus resistance traits introduced by conventional breeding, has resulted in increased weediness or invasiveness of a crop plant (Ref. 2).

Although virus infection has been shown to decrease growth and/or reproduction of some natural plant communities suggesting that acquired virus resistance has the potential to influence plant population dynamics (discussed below in Unit II.A.2), there are many reasons to believe the situation would be different for crop plants. Most naturalized, domesticated crops generally are unable to effectively compete with wild species in natural ecosystems and are unlikely to acquire this ability with genetic modification (Ref. 2). Plant breeders have capitalized for decades on the fact that relatively minor genetic changes can produce plants with altered ecological properties, but the addition of pest resistant traits has not been known to result in increased weediness of widely used crops, the possible exception being gene transfer from cultivated traditionally-bred sorghum to Johnson grass (Ref. 2). For domesticated crops, the traits that make them useful to humans also reduce their competitive ability in nonagricultural habitats. EPA believes crops that have been subjected to long-term breeding (e.g., corn, beans, maize, and wheat) are unlikely to possess characteristics that would allow the plant to compete effectively outside of managed ecosystems. Domesticity arises because many characteristics that would enhance weediness (e.g., seed shattering, thorns, seed dormancy, and bitterness) have been deliberately eliminated from the crop plant through intensive breeding efforts. For example, lack of seed shattering and seed dormancy greatly reduces the ability of an annual crop to persist without human intervention. Without major changes in its phenotype, corn is unlikely to survive for multiple generations outside agricultural fields no matter what transgene it contains (Ref. 3).

The reassuring history for cultivated plants does not completely preclude a crop containing a PVCP-PIP from becoming a weed, but it suggests that the likelihood of that event is small (Ref. 2). Given the selective disadvantage of crop plants in natural ecosystems, it is unlikely that acquisition of virus resistance would confer sufficient competitive advantage on naturalized populations of crops to upset the network of existing ecological relationships given that many other factors appear to constrain their competitiveness in non-cultivated areas (Ref. 1). A survey of the weedy characteristics of crop versus weed species showed that weeds possess significantly more weedy characteristics on average than do crop plants, suggesting that acquisition of any single trait would be insufficient to make a crop plant a weed (Ref. 4).

Thus, EPA believes that the available evidence supports a finding that there is a low probability of risk that a PVCP-PIP would cause the engineered crop plant to become wild or weedy. As a result, EPA believes that the only condition on an exemption that is necessary to ensure that crop plants containing PVCP-PIPs that qualify for exemption present only a low risk of disrupting ecological relationships is a requirement that the crop plant is not itself already a weedy or invasive species.

2. *Likelihood that a crop plant containing a PVCP-PIP could disrupt ecological relationships through gene transfer*

As discussed above in Unit II.A.1, the available evidence suggests that there is only a low likelihood that a PVCP-PIP is likely to cause the transgenic crop plant containing it to become weedy or invasive. However, the question of whether gene transfer to naturally occurring plants in the agroecosystem could lead to such adverse outcomes is a more complicated question because it involves a much broader range of potential plants. The answer to this question depends first on the question of whether the transgenic crop plants could transfer a PVCP-PIP to other plant populations. This potential for transfer depends on the frequency of hybridization between domesticated species and their wild relatives. Hybridization is affected by the ability of plants to cross-pollinate which in turn is affected by their timing of reproductive viability and the proximity of the plants. Hybridization is also affected by the ability of pollen to fertilize recipient plants, develop into viable seeds, germinate, and grow into a viable adult (Ref. 5). In spite of these potential constraints, a survey of the world's most important crops suggests that spontaneous hybridization of domesticated plants with wild relatives appears to be a general feature across at least a portion of the geographic area over which each is cultivated (Refs. 6, 7).

The answer to whether virus infection limits the growth and/or reproductive ability of wild or weedy plant populations appears to be much more variable. In general, viruses appear to be pervasive in natural plant populations (Refs. 8, 9, 10, 11), although a comprehensive body of literature on the presence and effect of viruses in weed species is lacking. Some studies report that virus infection has little or no effect on the plants (e.g., see Refs. 10, 12). However, other studies have reported that infection reduces plant growth and/or fecundity. For example, tobacco leaf curl gemininivirus infection increases mortality and has significant negative effects on growth and seed output in plants from wild populations of *Eupatorium chinense* (Ref. 13), and geminivirus infection likewise decreases growth and reproductive output of *Eupatorium makinoi* (Ref. 14). Field experiments showed that wild cabbage plants (*Brassica oleracea*) infected with turnip mosaic potyvirus or turnip yellow mosaic tymovirus have reduced survival, growth, and reproduction (Ref. 15). Similarly, cucumber mosaic virus infection was found to reduce vegetative growth and flower production of purslane (*Portulaca oleracea*) (Ref. 16).

It is difficult to predict the actual impact on overall plant population dynamics that would result from acquisition of virus resistance by plants that are in some way negatively affected by virus infection. EPA is not aware of any study that has directly examined this question by purposefully freeing a weed species from virus infection and investigating the resulting population dynamics of infected versus uninfected plants. However, some studies show that virus infection can in some cases affect plant population dynamics, suggesting such impacts may also result if the population were subsequently freed from virus infection. For example, infection with alfalfa mosaic virus substantially diminished the ability of certain medic cultivars (*Medicago polymorpha*) to compete with other species such as capeweed (*Arctotheca calendula*), both directly by decreasing the competitive ability of infected plants, and indirectly by altering the proportions in which the species germinated (Ref. 17). In another example of virus infection affecting plant population dynamics, growth analysis of *E. makinoi* revealed that plants naturally infected with a geminivirus had significantly reduced stem growth and plant height, along with decreased flowering and survivorship. This study suggests that such negative fitness attributes have a significant impact on plant population dynamics in this species (Ref. 18).

Although relatively little research has been conducted regarding how plant population dynamics are influenced by virus infection, such results as described in the previous paragraph support the

premise that at least some plant populations acquiring virus resistance might be able to outcompete other species (Ref. 19) and/or spread to previously unsuitable habitat (Ref. 20). However, it has also been discussed that acquisition of virus resistance might decrease plant fitness. For example, barley yellow dwarf virus was found in at least one year to increase the fitness of its host *Setaria viridis* by approximately 25% (Ref. 21). Such results might be expected if the plants become more attractive to herbivores when not infected by viruses, as was found to occur for seedlings of *Kennedya rubicunda* (Ref. 12). In a coastal bushland experimental site, virus-free seedlings were grazed at twice the rate as those manually inoculated with Kennedya yellow dwarf virus due to increased palatability to herbivores. Such considerations may be important in evaluating effects on endangered/threatened species.

EPA believes that many PVCP-PIP/plant combinations are likely to pose a low risk of disrupting the existing network of ecological relationships in semi-managed or natural ecosystems given that multiple factors must be present, i.e., hybridization with a wild relative must occur, introgression of the gene must occur, and acquired virus resistance must confer an advantage (or disadvantage) to the recipient plant sufficient to alter plant population dynamics. Nevertheless, the research discussed above showing that viruses can in some cases affect plant population dynamics highlights the difficulty in drawing a general conclusion as to whether all PVCP-PIP/plant combinations are likely to pose a low risk of disrupting existing ecological networks. Virtually any crop could be modified to contain a PVCP-PIP, including less domesticated forage crops and trees, and such a wide range of crop plants will be associated with a concomitantly wide range of characteristics and behaviors. Ecosystems are highly complex and variable, and the factors that limit establishment of a given plant species can be subtle and are not well understood (Ref. 3). Consequently, EPA does not believe that the available body of evidence would currently support a definitive conclusion for all PVCP-PIPs that the potential transfer to wild or weedy relatives presents a low risk of altering the network of ecological relationships in semi-managed or natural ecosystems. Thus, one of the key challenges that EPA has faced is how to clearly describe for regulatory purposes those situations in which gene transfer of a PVCP-PIP would not likely alter existing ecosystem relationships.

Information currently available does not appear sufficient to describe any set of circumstances that would predict for the wide variety of possible PVCP-PIP/plant combinations whether introgression of the PVCP-PIP into a wild or weedy relative could change the population dynamics of the recipient plant. For example, it is not possible to predict *a priori* whether a possible fitness advantage that individual plants might acquire with a PVCP-PIP would lead to the plant population outcompeting other species. Whether population dynamics would be affected in a given circumstance is dependent on multiple, interacting factors. In some instances, a weight-of-evidence, case-by-case review of information such as experimental data might allow such a determination; however, general knowledge of factors likely to influence population dynamics cannot be readily used for regulatory purposes to develop a clearly understandable criterion suitable for a categorical exemption. Thus, although EPA would like to describe an objectively defined criterion that distinguishes those PVCP-PIP/plant combinations that are likely to change plant population dynamics from those that are not, EPA has concluded that this is not currently feasible. Instead, EPA is considering an exemption criterion related to concerns associated with gene flow based on other relevant factors. Paragraph (1) of criterion (a), a categorical exemption criterion for a subset of PVCP-PIPs, was developed based on the potential for the genetic material of a PVCP-PIP to flow to wild or weedy relatives. Paragraph (2) of

criterion (a), an exemption criterion conditional on Agency review, was developed based on characteristics of the plant containing the PVCP-PIP and characteristics of that plant's wild or weedy relatives. Each part of criterion (a) is discussed in more detail below.

a. Categorical exemption criterion

For the reasons articulated above, EPA does not believe it can develop a categorical exemption that is based on whether a PVCP-PIP/plant combination is likely to result in changes in the population dynamics of wild or weedy relatives. However, EPA believes that a criterion for a categorical exemption could be developed based on exposure potential, i.e., whether genetic material, including the PVCP-PIP, could flow from the engineered crop plant to related wild or weedy relatives. Essentially, this criterion would focus on the first of the three events that must occur for a PVCP-PIP potentially to alter the existing network of ecological relationships through gene flow: whether the plant containing the PVCP-PIP has wild relatives growing within its vicinity with which it can produce viable hybrids. Basing a regulatory criterion on this consideration does not mean the Agency fails to recognize that several events are necessary before existing networks of ecological relationships could be disrupted; rather it is an attempt to create a clearly understandable regulatory criterion suitable for an exemption.

In developing any categorical exemption for a subset of PVCP-PIPs in which a developer could self-determine whether the criteria were met, EPA seeks to identify those situations that clearly pose low risk with respect to gene transfer. Although the Agency recognizes that many events must occur before transfer of a PVCP-PIP would cause an adverse environmental impact, the inability of the crop plant to hybridize with wild or weedy relatives provides the most straightforward assurance in an objective criterion that an adverse environmental impact would not occur for a particular PVCP-PIP/plant combination.

A PVCP-PIP would meet criterion (a) under paragraph (1) if the plant containing the PVCP-PIP is one of the following: almond (*Prunus communis*), apricot (*Prunus armeniaca*), asparagus (*Asparagus officinale*), avocado (*Persea americana*), banana (*Musa acuminata*), barley (*Hordeum vulgare*), bean (*Phaseolus vulgaris*), black-eyed pea (*Vigna unguiculata*), cacao (*Theobroma cacao*), celery (*Apium graveolens*), chickpea (*Cicer arietinum*), citrus (*Citrus* spp.), coffee (*Coffea arabicua*), corn (*Zea maize*), cucumber (*Cucumis sativus*), eggplant (*Solanum melongena*), guava (*Psidium guajava*), kiwi (*Actinidia* spp.), mango (*Mangifera indica*), nectarine (*Prunus persica*), okra (*Abelmoschus esculentus*), olive (*Olea europaea*), papaya (*Carica papaya*), parsley (*Petroselinum crispum*), pea (*Pisum sativum*), peach (*Prunus persica*), peanut (*Arachis hypogaea*), pineapple (*Ananas comosus*), pistachio (*Pistacia vera*), plum (*Prunus domestica*), potato (*Solanum tuberosum*), soybean (*Glycine max*), spinach (*Spinacia oleracea*), starfruit (*Averrhoa carambola*), taro (*Colocasia esculenta*), tomato (*Lycopersicon lycopersicum*), or watermelon (*Citrullus lanatus*).

Plant species on this list were identified by the October 2004 FIFRA SAP as having no wild or weedy relatives in the United States, its possessions, or territories with which they can produce viable hybrids in nature. Thus, given the extremely low probability that virus resistance could be transferred to another species, any PVCP-PIP contained in such a plant would pose a low

probability of altering existing plant population dynamics or existing ecological relationships. In addition, a list is very straightforward, providing an easy-to-understand criterion. EPA thus believes that a developer could self-determine eligibility as no further data or information would be needed to evaluate whether ecological relationships could be disrupted through gene flow when the plant containing the PVCP-PIP is on the list.

The SAP noted (Ref. 22) that some of the plants on this list were able to escape cultivation and form occasional volunteer populations (i.e., asparagus and celery). Upon further investigation, EPA determined that other plant species on the list are also able to naturalize in some region of the United States, its possessions, or territories (e.g., soybean and corn). The ability to naturalize is an apparently common feature of crop plants (see www.plants.usda.gov). As EPA has previously discussed, naturalized populations of most crop plants, particularly those recently establishing such populations, would be expected to carry with them a suite of traits suitable to cultivation in a managed habitat but that confer a selective disadvantage on plants in the wild. For these crops, the traits that make them useful to humans also reduce their competitive ability in nonagricultural habitats (see Unit II.A.1).

b. Exemption criterion conditional on Agency determination

The Agency recognizes that many PVCP-PIPs would reasonably be expected to pose low risk with respect to potential for disturbing existing ecological relationships among plants even though the crop plant containing the PVCP-PIP is not on the above list. In such cases, although there exists the potential of the plant containing the PVCP-PIP to hybridize with wild or weedy relatives in some region of the United States, its possessions, or territories, additional events are likely necessary for any adverse environmental outcomes to occur, e.g., acquired virus resistance must confer an advantage (or disadvantage) on the recipient plant sufficient to alter plant population dynamics. As discussed above in Unit II.A.2, given the diversity of plants that could contain a PVCP-PIP and the complex and variable nature of ecosystems, EPA's challenge is to develop an objectively defined criterion that would describe for regulatory purposes only those PVCP-PIP/plant combinations that would likely not significantly disrupt natural plant population dynamics. However, developing such a criterion may currently not be feasible because of insufficient information to make generic determinations regarding characteristics of a plant and/or PVCP-PIP that would indicate such events are unlikely to occur.

EPA does not believe it can develop at this time a broader categorical exemption criterion than that discussed above (Unit II.A.2.a) which allows developers to self-determine whether their PVCP-PIP/plant combination meets the criterion. However, by relying on a case-by-case Agency determination of whether the PVCP-PIP/plant combination meets a criterion, EPA might be able to expand any exemption to include a larger set of PVCP-PIP/plant combinations expected to present low risk. Nevertheless, even with an Agency determination, EPA must still define a criterion with sufficient precision such that the public would be able to understand what products would qualify and evaluate whether they meet the standard for a FIFRA exemption. Such a criterion is difficult to develop because many characteristics would influence this determination in ways that could only be poorly defined for the entire class of PVCP-PIPs. In addition, many relevant considerations such as the impact of virus infection on wild plant populations and the

likely selective advantage afforded by acquisition of virus resistance are currently poorly understood for the vast majority of species. The scarcity of research in this area makes it particularly difficult to construct criteria describing low risk groups with sufficient precision such that a product developer or the public would be able to effectively determine whether a product would qualify for the exemption.

Nonetheless, in addition to the categorical exemption for a subset of PVCP-PIPs discussed above in Unit II.A.2.a, EPA also believes that a criterion conditional on Agency determination could be developed based on whether the transgenic plant itself is a weedy or invasive species or whether gene flow could occur from the transgenic plant to a weedy or invasive species or endangered/threatened species. Given that such plants already are associated with serious environmental concerns, any disruption of their population dynamics could have significant consequences. EPA has therefore determined that PVCP-PIPs that are in or could potentially end up in such plants through gene flow would have to go through the registration review process. Although the Agency recognizes that transfer of a PVCP-PIP into a nearby relative that is not a weedy or invasive species also has the potential for altering plant population dynamics, the changes in such circumstances are unlikely to rise to the level requiring the regulation provided by the registration process because the plant population acquiring the virus resistance trait is not already weedy or invasive. Species composition of natural communities is likely a dynamic variable, constantly changing in response to diverse environmental factors. The changes in plant population dynamics potentially introduced when a plant that is not weedy or invasive acquires virus resistance are likely to be within the range of changes that happen naturally within plant communities without adverse effects.

Accordingly, EPA is considering an approach under which PVCP-PIP/plant combinations that fail to meet paragraph (a)(1) could still meet criterion (a), subject to an Agency review to determine whether they meet a different set of conditions related to this issue. Under such an approach, a PVCP-PIP would meet criterion (a) under paragraph (2) if the Agency determines that the plant containing the PVCP-PIP (i) is itself not a weedy or invasive species outside of agricultural fields in the United States, its possessions, or territories, and (ii) does not have relatives outside of agricultural fields in the United States, its possessions, or territories that are weedy or invasive species or endangered/threatened species with which it can produce viable hybrids in nature.

Under such an approach, PVCP-PIPs could qualify for exemption if they are in a plant species that is not a weedy or invasive species and does not have relatives that are weedy or invasive species based on the low probability that acquisition of a virus-resistance trait would confer sufficient additional competitive advantage on plants that are not already weedy or invasive to lead to adverse environmental outcomes. Even in cases when a plant population is under intense disease selection pressure, this selective pressure is unlikely to be the only condition restraining the population. It is unlikely that the use of PVCP-PIPs would affect wild or weedy relatives differently than what has occurred in the past with virus resistant varieties developed through traditional breeding and grown throughout the United States over many years. The source of resistance traits in such conventionally bred crops is often a wild relative of the crop. There is no indication that growing such crop plants near wild or weedy relatives has resulted in these relatives becoming any more of a weed problem than they were previously due to acquisition of the virus-resistant trait (Ref. 2).

In addition, outbreeding depression between crop plants and their wild relatives appears to be more common than hybrid vigor (Ref. 23). In outbreeding depression, mating between individuals from two different environments can disrupt gene combinations that are favored by natural selection in each environment. Resulting offspring may have phenotypes that are poorly adapted to the habitat of either parent. Thus, hybrid offspring acquiring a PVCP-PIP are often likely to be less competitive than their wild parent in nature. While this observation supports the contention that crop-wild hybrids containing a PVCP-PIP are unlikely to outcompete other plants, it also highlights the hazard crop-wild hybridization may pose to endangered/threatened species. EPA thus addresses endangered/threatened species in criterion (a)(2) to ensure that a PVCP-PIP would not exacerbate population loss in such species.

When EPA asked the FIFRA SAP in 2004 about the likelihood that plant populations freed from viral pressure could have increased competitive ability leading to changes in plant population dynamics, the FIFRA SAP offered the following opinion: "[b]ased on knowledge obtained from observation of cultivated crops in the agroecosystem, the majority of the [2004] Panel concluded that it would be unlikely that a plant population freed from viral pressure would give a plant species a competitive advantage" (Ref. 22).

EPA means by the term "weedy or invasive species" plants that are: (1) either non-native (or alien) to the ecosystem under consideration or aggressive competitors in their natural ecosystems, and (2) whose introduction causes or is likely to cause economic or environmental harm or harm to human health. EPA considers a non-native (or alien) species to be synonymous with an introduced species, or one that occurs in a region in which it is not native. EPA uses the terms endangered species and threatened species consistent with their meaning under the Endangered Species Act.

During its review, EPA would consider the most recent scientific information about the plant species containing the PVCP-PIP and its wild or weedy relatives to evaluate the potential for weedy or invasive behavior, including whether any of these species are extending their range. The Agency would evaluate a number of sources including existing lists of invasive weeds, e.g., the Federal Noxious Weed List. Examples of plants related to crop species that generally are considered to be weedy or invasive species by a number of organizations are animated oats (*Avena sterilis*), johnsongrass (*Sorghum halepense*), red rice (*Oryza punctata*), wild safflower (*Carthamus oxyacantha*), and wild sugarcane (*Saccharum spontaneum*). Inclusion on any given list would be informative, but not determinative for the Agency's evaluation. Examination of existing lists has shown that different organizations use different criteria for listing species depending on the goals and missions of those organizations. Thus the Agency would use existing lists as a resource much as it would use published literature, rather than as determinative sources. For example, plants that may form volunteer populations in agricultural fields are considered weeds by some organizations and may appear on those organizations' weed lists, but for reasons described above in this Unit, EPA would not consider propensity to volunteer, i.e., to grow in a field from seeds dropped from the previous crop rotation, to be indicative of general weediness potential for a plant. EPA would include consideration of whether the plant is invasive or weedy outside of agricultural fields to emphasize that the key consideration is the plant's behavior in natural settings, including semi-managed habitat surrounding agricultural fields as opposed to its behavior within the fields themselves.

EPA does not intend to develop or maintain its own list of weedy and invasive species. Plants are regularly being newly classified as weedy or invasive by various weed societies and other organizations as more information is acquired and as plants extend their ranges. Given the difficulties associated with developing and maintaining a comprehensive list and the many considerations comprising weediness or invasiveness, the Agency believes that individual case-by-case determination for each plant would be preferable.

For the purposes of paragraph (a)(2), EPA would focus on whether the recipient plant "can produce viable hybrids in nature" because the Agency believes that this characteristic is a critical attribute that would determine the potential for introgression of the PVCP-PIP into a native or naturalized plant population. Although hybrids must be able to reproduce themselves in order for introgression to occur, the Agency has chosen to focus on the production of "viable" hybrids (i.e., those that are able to grow) because this characteristic may be described more clearly in a regulatory standard than examining the reproductive potential of any hybrids. In many cases, reproductive potential of hybrids has not been fully investigated. Given that reduced fertility in F1 crop-wild hybrids is frequently restored to normal in subsequent generations (Ref. 20), measurement of hybrid fertility involves consideration of several generations. In addition, viability is the appropriate standard because even very low rates of gene transfer can lead to introgression (Ref. 24), suggesting that any degree of hybrid fertility could indicate the potential for introgression to occur. The Agency recognizes that introgression of a trait such as virus resistance into natural plant populations does not automatically confer a competitive advantage to the recipient population. However, at this time, there is little information available to predict categorically whether acquisition of such a trait might affect the competitiveness of a specific plant population, and the available information does not allow the Agency to make this determination *a priori*. EPA therefore relied on the ability to produce viable hybrids when developing this criterion. The language also clarifies that the relevant question is whether the hybrid can be produced "in nature." The fact that plants could be crossed in the laboratory is not necessarily indicative of a plant's true reproductive potential. The Agency's focus would be on whether a viable hybrid could be produced under normal growing conditions in the field or in nature, rather than under controlled experimental conditions that might have little relevance to how the product would behave in the environment.

For the purposes of paragraph (a)(2), EPA is considering whether to limit the exemption to those PVCP-PIPs present in plant that is itself not a weedy or invasive species in the United States, its possessions, or territories and does not have relatives in the United States, its possessions, or territories that are weedy or invasive species or endangered/threatened species. The Agency believes the entire United States, including its possessions and territories is the relevant scope of inquiry because an exemption would carry no limitations on where the exempted PVCP-PIP plant combination could be planted and thus could be planted in all areas subject to U.S. law. FIFRA section 2(aa) defines "State" as "a State, the District of Columbia, the Commonwealth of Puerto Rico, the Virgin Islands, Guam, the Trust Territory of the Pacific Islands, and American Samoa.

The Agency's rationale for excluding from exemption plants that fail to meet criterion (a)(2) would be the recognition that weedy and invasive species are already associated with significant economic costs and ecological damage. For plants that fail to meet criterion (a) and thus do not qualify for exemption, an applicant may apply for a registration under section 3 of FIFRA.

During review of the registration application, in addition to other considerations, the Agency may evaluate whether a plant's weedy or invasive characteristics could be augmented by acquisition of a virus resistance trait. A case-by-case review for registration would allow the Agency to evaluate in depth the potential impacts of acquisition of a PVCP-PIP by considering, for example, the effect of virus infection on such species, the existence and impact of any natural virus resistance in the population, the overlap of the plant's distribution with crop cultivation areas, and other relevant traits. As part of registration, the Agency could also impose control conditions if possible and appropriate.

c. Other approaches

In 1994 EPA proposed two different alternatives for exempting PVCP-PIPs from FIFRA requirements. Although the Agency is still considering these options, they are no longer the Agency's preferred approach for a number of reasons. One of these options contained criteria directed towards addressing concerns associated with gene transfer. Under this alternative, the Agency defined a set of criteria to identify those PVCP-PIP/plant combinations with the lowest potential to confer selective advantage on wild or weedy plant relatives. Only those PVCP-PIPs so identified would have been exempt from regulation under the 1994 proposal. In 1994 EPA described this alternative exemption as follows:

> "Coat proteins from plant viruses [would be exempt] if the genetic material necessary to produce a coat protein is introduced into a plant's genome and the plant has at least one of the following characteristics:
>
> "(1) The plant has no wild relatives in the United States with which it can successfully exchange genetic material, i.e., corn, tomato, potato, soybean, or any other plant species that EPA has determined has no sexually compatible wild relatives in the United States.
>
> "(2) It has been demonstrated to EPA that the plant is incapable of successful genetic exchange with any existing wild relatives (e.g., through male sterility, self-pollination).
>
> "(3) If the plant can successfully exchange genetic material with wild relatives, it has been empirically demonstrated to EPA that existing wild relatives are resistant or tolerant to the virus from which the coat protein is derived or that no selective pressure is exerted by the virus in natural populations" (59 FR 60504).

EPA carefully reconsidered this 1994 proposal in its deliberations and presented several modified criteria to the FIFRA SAP at the October 2004 meeting for consideration. In light of comments received from the FIFRA SAP and additional scientific information available since 1994, EPA no longer believes this alternative would adequately address questions associated with weediness in a manner that could be reasonably implemented. However, EPA still considers that it would be appropriate to limit an exemption based on the concerns outlined in the earlier proposal associated with acquisition of virus resistance through hybridization with a transgenic plant containing a PVCP-PIP.

Although similar in intent to the first characteristic of this option proposed in 1994, criterion (a) in this document focuses on the potential to "form viable hybrids in nature" rather than simply "exchange genetic material" because the former is more critical for determining whether a

PVCP-PIP might negatively affect a recipient plant population. The ability to exchange genetic material, which is often demonstrated by performing hand crosses in the laboratory, may not indicate any relevant information about how the plants would behave in nature. The approach the Agency is currently considering also expands the list of plants meeting this condition beyond the four in the 1994 proposal. When EPA presented a similar criterion to the 2004 SAP, they responded that "the Panel was of the opinion that the absence of a competent wild/weedy relative positioned in relation to the plant containing the PVCP-PIP was an appropriate condition" (Ref. 22).

EPA now also believes that the second characteristic of the option proposed in 1994 may be insufficient based on the conclusions of the 2004 SAP that current methods of bioconfinement are imperfect and are unlikely to adequately restrict gene flow (Ref. 22). The Agency asked whether the condition that "genetic exchange between the plant into which the PVCP-PIP has been inserted and any existing wild or weedy relatives is substantially reduced by modifying the plant with a scientifically documented method, (e.g., through male sterility)" would be necessary and/or sufficient to minimize the potential for a PVCP-PIP to harm the environment through gene transfer from the crop plant containing the PVCP-PIP to wild or weedy relatives. The Panel "accepted that tactics aiming at diminished gene exchange are highly desirable and even necessary but are not sufficient." EPA believes that criterion (a) in the approach the Agency is currently considering more precisely defines those situations where successful genetic exchange is either not possible (under paragraph (1)) or not of concern (under paragraph (2)). However, EPA is still considering whether it would be possible to construct a criterion involving significantly reduced potential for gene exchange such as that presented to the 2004 SAP that would enable the Agency to determine with review that a product presents low risk with respect to concerns associated with gene flow.

EPA believes that the third characteristic of the option proposed in 1994 is sound conceptually, but impractical to implement in an exemption. Appropriate and generically applicable protocols that could be followed to demonstrate convincingly that either condition listed in characteristic (3) was met are unavailable. In particular, generic sampling protocols are especially difficult to develop given that plant species are extremely diverse, e.g., in geographic distribution and life history. Based on subsequent consideration, the Agency presented the following revision to the 2004 SAP for their consideration: "all existing wild or weedy relatives in the United States with which the plant can produce a viable hybrid are tolerant or resistant to the virus from which the coat protein is derived." Among the Panel members, "[i]t was generally accepted that such wording was not helpful for a number of reasons." Specifically, "[t]he Panel had particular difficulty when attempting to add precision to approaches that should be followed when sampling wild and weedy relatives for the occurrence of specific virus tolerance or resistance as specified by the Agency." However, the Agency still recognizes the scientific utility of evaluating the impact of virus infection on wild or weedy plant populations that could acquire a PVCP-PIP through gene flow when attempting to determine whether a PVCP-PIP presents low risk. If such a criterion could be clearly articulated such that the public and product developers would have a reasonable understanding of which PVCP-PIPs would qualify for exemption and which would not, the Agency would consider incorporating this concept into an exemption.

The other option proposed in 1994 did not contain a criterion addressing concerns associated with gene flow. This option proposed a full categorical exemption for all PVCP-PIPs (59 FR

60503). Although the Agency is still considering this option, it is no longer the Agency's preferred approach for a number of reasons. Specifically, EPA has received scientific advice since the 1994 proposal calling into question the Agency's 1994 rationale that all PVCP-PIPs meet the FIFRA 25(b)(2) exemption standard, including gene flow considerations. Although EPA believes that many PVCP-PIPs present low risk and thus meet the FIFRA 25(b)(2) exemption standard, in order to categorically exempt all PVCP-PIPs, the Agency must be able to draw this conclusion for all PVCP-PIPs. Advances in scientific understanding since 1994 suggest it may not be possible to support this rationale for all PVCP-PIPs and that certain PVCP-PIPs may pose a greater level of risk than is characteristic of the group as a whole. For example, virus resistance is common in natural plant populations as evidenced by conventionally-bred virus resistant plants that are only possible due to existing resistance in available breeding stock (Ref. 25). This fact suggests that acquisition of virus resistance is often unlikely to introduce a novel trait into many plant populations. However, some notable exceptions to the ubiquity of virus resistance in natural plant populations exist including the lack of successful conventionally bred resistance to barley yellow dwarf virus in major crops and the lack of natural resistance in some wild relatives of these crops (Ref. 19). Such information suggests that acquisition of a PVCP-PIP by such wild relatives of these plants has the potential to free these wild relatives from what may be an important ecological constraint. The conclusions of the 2004 FIFRA SAP are consistent with the idea that it may not be possible to apply a general exemption rationale to all PVCP-PIPs. The report concluded that "…PVCP-PIPs [have] no inherent capacity to harm the environment." However, "[i]t was recognized that knowledge of hybridization potential was sparse and of very unequal quality but the likelihood of serious economic harm was such that some plants engineered to contain stress tolerant traits should not be released" (Ref. 22). Similarly, the 2000 National Research Council (NRC) report recommended that because of concerns associated with outcrossing to weedy relatives, "EPA should not categorically exempt viral coat proteins from regulation under FIFRA. Rather, EPA should adopt an approach, such as the agency's alternative proposal…, that allows the agency to consider the gene transfer risks associated with the introduction of viral coat proteins to plants" (Ref. 26).

B. Viral Interactions

A key concern associated with PVCP-PIPs is the question of whether they could affect the epidemiology and pathogenicity of plant viruses. Given the potential impact of virus infection, such changes might affect competitiveness of plant populations thereby altering ecosystem dynamics, e.g., through changes in species composition of populations, resource utilization, or herbivory.

Mixed viral infections are extremely common in crops and other plants (Ref. 27). In natural, mixed infections, viral genomes from different strains and/or different species simultaneously infect the same plant and thus have opportunities to interact (e.g., through recombination, heterologous encapsidation, or synergy). In spite of many opportunities for interaction in nature, such events rarely lead to any detectable adverse outcome (Ref. 28). However, such *in planta* interactions do have the potential to result in a virus that causes increased agricultural or other environmental damage. For example, the epidemic of severe cassava mosaic disease in Uganda is thought to be due to the combination and/or sequential occurrence of several phenomena

including recombination, pseudorecombination, and/or synergy among cassava geminiviruses (Ref. 29).

In transgenic plants containing PVCP-PIPs, every virus infection is essentially a mixed infection with respect to the coat protein gene (Ref. 30). The key question facing EPA is whether interactions between such introduced plant virus sequences and other invading viruses in transgenic plants may increase in frequency or be unlike those expected to occur in nature (Ref. 31). The issues associated with recombination, heterologous encapsidation, and synergy are briefly described below. EPA provides a general overview of each of the processes separately, followed by a brief review of relevant field studies that investigated these processes.

1. Recombination

Recombination is a natural process that can occur during replication of DNA or RNA whereby new combinations of genes are produced. Plant virus recombination can occur between members of the same virus pathotype in natural infections, contributing to the number of variants that exist within that pathotype. Recombination can also occur when different viruses coinfect the same plant and interact during replication to generate virus progeny that have genetic material from each of the different parental genomes. Although recombination likely occurs regularly in mixed viral infections, recombination only rarely leads to viable viruses with truly novel behavior and/or characteristics or any detectable adverse outcome. In order to persist in the field, a recombinant virus must compete with variants of the parental viruses that are already highly adapted to existing conditions, in all stages of the infective cycle, for example in transmission, gene expression, replication, and assembly of new virions (Ref. 28). An analysis of cucumber mosaic virus (CMV) isolates in natural populations showed that viable recombinants were very rarely recovered in mixed infections (Ref. 32).

However, laboratory experiments suggest that viruses with increased pathogenicity or altered epidemiology can be created through recombination. A pseudorecombinant strain created by experimentally combining regions of the CMV and tomato aspermy cucumovirus (TAV) genomes was found to have more severe symptoms than either of the parental genomes, although the recombinant wasn't able to move beyond infection of the initially infected cells (Ref. 33). Experiments have also shown interspecific recombination between CMV and TAV under conditions in which recombinants would not be expected to have any particular fitness advantage (Ref. 34). In another example, alteration of the host range of tobacco mosaic virus (TMV) occurred when a chimeric virus expressed the coat protein from alfalfa mosaic virus (AMV) instead of its own (Ref. 35).

Moreover, even though selection in the field appears to act against persistence of a new recombinant virus, recombination is thought to play a significant role in virus evolution. Evidence of past recombination having led to the creation of new DNA and RNA viruses has been documented in a number of different groups including bromoviruses (Ref. 36), caulimoviruses (Ref. 37), luteoviruses (Ref. 38), nepoviruses (Ref. 39), cucumoviruses (Ref. 40), and geminiviruses (Refs. 29, 41). Sequence analysis of viruses from the family Luteoviridae indicated that this family has evolved via both intra- and interfamilial recombination (Ref. 42).

Several instances can be cited in which relatively recent recombination events appear to have resulted in the creation of new viruses. For example, numerous recombination events among tomato-infecting begomoviruses around the Nile and Mediterranean Basins are likely at least partially responsible for numerous whitefly-transmitted tomato diseases that have emerged in the last 20 years (Ref. 43). In addition, a natural recombinant between Tomato yellow leaf curl Sardinia virus and Tomato yellow leaf curl virus was detected in southern Spain with a novel pathogenic phenotype that might provide it with selective advantage over the parental genotypes (Ref. 44). Finally, analysis of a newly described *Curtovirus* species associated with disease of spinach in southwest Texas suggests that it may be the result of recombination among previously described *Curtovirus* species (Ref. 45).

In addition to virus-virus recombination, recombination has also been found to occur between virus and plant host RNA. Sequence analysis of the 5' terminal sequence of potato leafroll virus (PLRV) suggests that it arose via recombination with host mRNA (Ref. 46). Evidence suggests that such recombination events can affect virus virulence (for review see Ref. 47). Several experiments have therefore investigated whether a PVCP-PIP integrated into a plant genome is able to recombine with the genetic material of an infecting virus. Like a plant host genome, viral transgenes would be available for recombination with infecting viruses, and portions of the transgene could thus be incorporated into the replicating virus. Laboratory experiments with pseudorecombinant transcripts of papaya ringspot virus (PRSV) have shown that recombinant viruses that theoretically could be produced in field-grown transgenic papaya would be able to affect the virulence of the infecting strains (Ref. 48).

Several laboratory experiments have also investigated the potential for recombination between viral transgenes and infecting viruses of the same species. These experiments show that recombination can occur between viral transgenes and both RNA viruses (Refs. 49, 50, 51, 52, 53) and DNA viruses (Refs. 54, 55, 56, 57). However, the transgenic plants used in these DNA virus experiments actually show no viral resistance; attempts to develop transgenic DNA virus-resistant plants in general have had little success (Ref. 27). In addition, to facilitate the detection of recombinants, most of these experiments were conducted under conditions of high selective pressure favoring the recombinant, i.e., only recombinant viruses were viable. The selective pressure under normal field conditions would likely favor the parental viruses rather than a recombinant as parental viruses will outnumber the new recombinant and will be competent in all of the functions needed for propagation.

The above information suggests that recombination among viruses likely leads to rare instances of adverse changes in virus epidemiology and/or pathogenicity. Based on the available information, EPA is not able to rule out the concern that viable recombinant viruses could arise in plants containing a PVCP-PIP. The body of existing scientific knowledge supports the contention that recombination between a PVCP-PIP and an infecting virus could lead to environmental impacts in some instances. However, the vast majority of such interactions are expected to be no different from those that would occur in a natural mixed infection of the respective viruses and would not cause any adverse environmental effects beyond what could occur in the absence of the PVCP-PIP. EPA believes that the Agency has identified in this discussion those few circumstances in which the potential recombinants involving the PVCP-PIP could involve viruses that would otherwise not be expected to interact in a mixed infection found in nature (i.e., leading to "novel viral interactions") and for which additional data or information

would therefore be needed to make a determination that the PVCP-PIP could qualify for exemption from regulation under FIFRA.

2. Heterologous encapsidation

Heterologous encapsidation occurs when the coat protein subunits of one virus surround and encapsidate the viral genome of a different virus. The coat protein, possibly in conjunction with other viral factors, is essential for transmission and responsible for conferring the high degree of vector specificity. Therefore, a heterologously encapsidated viral genome may be transmitted by the vectors of the virus contributing the coat protein rather than the vectors of the virus contributing the viral genome. For many viruses, transmission from plant to plant occurs by insect vectors, and each virus tends to be transmitted by only one type of insect (Ref. 58). To the extent that vectors visit different groups of plants, vectors carrying a heterologously encapsidated viral genome may carry it to a plant it does not normally encounter (Ref. 30).

Most evidence of heterologous encapsidation is derived from laboratory or greenhouse studies. The high frequency of mixed infections suggests the potential for heterologous encapsidation to occur in nature is great, but most mixed infections do not lead to heterologous encapsidation, and those virus interactions that do occur are very specific (Ref. 59). Heterologous encapsidation is however known to be a regular occurrence among some plant viruses. Its frequency depends on the viruses involved and is more likely to occur among close relatives (Ref. 60). An expansion of aphid vector specificity due to heterologous encapsidation was first observed in plants infected with two different isolates of barley yellow dwarf luteovirus (BYDV; Ref. 61) and was later shown to be a general phenomenon among these viruses in natural populations of several plant species (Ref. 62). Heterologous encapsidation was also shown to occur in potyviruses. An isolate of zucchini yellow mosaic virus (ZYMV) that is normally non-aphid transmissible due to a transmission-deficient coat protein was found to be transmitted by the aphid vector due to heterologous encapsidation when in a mixed infection with another potyvirus, papaya ringspot virus (Ref. 63). Heterologous encapsidation may sometimes be an important route of disease transmission. For example, umbraviruses do not encode a coat protein, and therefore transmission between plants occurs through encapsidation by an aphid-transmissible luteovirus coat protein (Ref. 64).

Heterologous encapsidation is considered a possible environmental concern because of the potential that a virus may be spread to plants it ordinarily had no means of reaching and thus could not have infected. Such concerns are largely mitigated by several factors. First, the heterologously encapsidated viral genome may not be able to replicate in the new host plant and could therefore not actually infect it. Second, if replication is possible in the new plant, the replicating viral genome would produce its own coat protein rather than that which heterologously encapsidated it. This virus would not be transmitted by the new vector which brought the heterologously encapsidated nucleic acid to the plant. The epidemiological consequences of such heterologous encapsidation would thus be limited. Another consideration is that for some viruses, effective vector transmission may depend on more than the coat protein (Ref. 65), requiring other regions of the viral genome, e.g., coat protein read-through domains or helper factors, and a PVCP-PIP producing other viral proteins would not qualify for the

exemption discussed here. Thus, in such cases heterologous encapsidation would not lead to a change in vector specificity. Finally, in large monocultures of crop plants, a vector is most likely to transmit even a heterologously encapsidated virus to the same plant that the virus is already able to infect (Ref. 65).

EPA has evaluated a number of circumstances to determine whether heterologous encapsidation might nevertheless be of environmental concern. For example, EPA considered whether a virus that is heterologously encapsidated and carried to a new host plant might be exposed to a vector that feeds on the new host plant and perhaps other plants the virus ordinarily could not access. EPA considered whether this new vector might in some cases be able to transmit the virus even though it would be encapsidated in its own coat protein, thereby expanding the virus' vector range. A new vector could possibly transfer the virus to new host plants, thus expanding the plant host range as well (Ref. 27). EPA considers expansion of host range through heterologous encapsidation to be an extremely unlikely outcome because such an outcome depends on each event in a series of rare events occurring. Should the probability of occurrence of any one event in this series be zero, the adverse event of an expanded host range would not occur. First, a virus must be heterologously encapsidated, an event that is not possible for every viral genome-coat protein combination. Second, the encapsidated viral genome must be transmitted by a new vector. Third, the transmission must be to a new host plant. Fourth, the heterologously encapsidated viral genome must be able to replicate in the new host plant. Fifth, the resulting virus, now encapsidated in its own coat protein, must be exposed to a new vector the virus never encountered before that is nevertheless able to transmit it. Finally, the virus must be transmitted by this vector to a new plant that the virus' prior vectors never visited. For such a series of events to be novel, the viruses, vectors, and plants involved must have had no previous opportunity to interact, but this requirement is rarely met. For example, it is known that many viruses are transmitted by polyphagous insects, which would facilitate introduction of the virus to many potential hosts (Ref. 27), and viruses may be transmitted at low frequency by a range of species other than their primary vector or mechanically, e.g., through the practices of modern agriculture (Ref. 66).

Another scenario EPA considered is that with a high enough frequency of vector transmission to a new host plant due to heterologous encapsidation, secondary spread among new plant hosts might not be required for the phenomenon to affect the population, assuming that the virus is able to decrease the new host plant's growth and/or reproduction. Although this scenario may be more likely to occur than an expansion of host range given that fewer rare events would have to occur, any impact on the affected plant population would be highly localized being confined to plants in or near transgenic crop fields. Such negative impacts are unlikely to be sufficiently detrimental to require FIFRA regulation given their localized nature and the probability that common agricultural practices (e.g., vector control) could be used to manage the problem. Moreover, although isolated instances of transmission may occur, a significant proportion of a plant population is unlikely to be infected in such a scenario. For example, a field experiment (discussed in Unit II.B.4) showed that heterologous encapsidation led to infection of only 2% of plants compared to 99% of plants infected under similar conditions by a virus that is not heterologously encapsidated (Ref. 67). Most importantly, the heterologously encapsidated virus will still have no way to spread among or beyond the plants of the affected population. In the case where a plant population contains relatively few individuals such that the impact of single plant infections would be magnified, plant infections are even less likely to occur because in

addition to the inefficient nature of heterologous encapsidation, the vector would be less likely to feed on a rare plant and more likely to feed on the more abundant transgenic crop plants. In some cases a vector may have a strong preference for a specific plant over even closely related plants (Ref. 68).

Finally, EPA considered that after expansion to a new host, rapid selection of variants best adapted to the new environment might lead to the evolution of a new virus (Ref. 27). However, all viruses that are occasionally heterologously encapsidated and transmitted to a new plant host have had the opportunity to adapt to new plant environments. The opportunities for rapid viral evolution presented by transgenic plants containing PVCP-PIPs would not, under any reasonably likely circumstances, be fundamentally different from what occurs in nature because it is not dependent on the unique combination of viruses that interact but rather the occurrence of a virus in a new plant host, an event that likely occurs in nature at some frequency for most viruses either through heterologous encapsidation or through occasional transmission that occurs mechanically or from secondary vectors (Ref. 66).

Experimental studies have shown that the PVC-protein in transgenic plants has the ability to encapsidate even unrelated infecting viruses (Refs. 69, 70, 71, 72). However, heterologous encapsidation involving a viral transgene can only occur if it expresses coat protein that possesses the appropriate physical parameters to encapsidate the viral genome of infecting viruses. In transgenic VCP plants that express very little coat protein (i.e., those relying on post-transcriptional gene silencing to confer resistance), the probability of heterologous encapsidation would be very small except in cases of suppression of gene silencing. (For a more detailed discussion of post-transcriptional gene silencing and suppression of gene silencing, see Unit II.B of Appendix II: Draft Approach to Exempting PVC-Proteins from the Requirement of a Tolerance under FFDCA.) In addition, as with recombination, as long as the VCP inserted in the transgenic plant is from a virus that normally infects the plant in the area where it is planted, the outcome of any heterologous encapsidation that may occur is expected to be the same in transgenic plants as in natural, mixed infections.

3. Synergy

In synergy, another type of viral interaction, the disease severity of two viruses infecting together is greater than expected based on the additive severity of each virus alone. For example, when a plant containing potato virus X (PVX) is coinfected with a number of potyviruses including tobacco vein mottling virus, tobacco etch virus, and pepper mottle virus, the disease symptoms are considerably worsened and PVX accumulates to a greater concentration (Ref. 73). A listing of reported viral synergisms has been compiled (Ref. 74).

The question EPA must address in developing an exemption is whether an infecting virus might have increased disease severity when infecting a plant containing a PVCP-PIP. For this to occur, the PVC-protein must be at least one of the factors causing synergy. However, the coat protein is considered much less likely to be responsible for synergism than other parts of the virus (Refs. 75, 76), and a PVCP-PIP producing other viral proteins would not qualify for the exemption under consideration here. In addition, any negative effects are expected primarily in the

transgenic crop itself, as expression of the coat protein would be necessary to produce the synergistic disease. Furthermore, any negative effects are expected to be self-limiting because any plants containing a PVCP-PIP that is prone to display synergy with viruses common in the areas of planting would be quickly abandoned once such effects were detected, perhaps as early as the field-testing stage of product development. Synergistic interactions can be evaluated in transgenic plants before deployment by experimental inoculation with all of the viruses likely to be encountered in the field (Ref. 65). Developers have a strong incentive to undertake such efforts to ensure the efficacy of their product after deployment.

4. Field experiments

The experiments referenced in Units II.B.1-3 above investigated potential viral interactions in VCP-transgenic plants under laboratory conditions. However, equally important is consideration of the likelihood and potential impact of viral interactions under natural field conditions (Ref. 77). Relatively few field studies have been conducted to address the questions EPA is evaluating, but the Agency has carefully considered the available literature.

A six-year experiment searched for and failed to find evidence of interactions involving viral transgenes in 25,000 transgenic potato plants transformed with various PLRV coat protein constructs. Plants were exposed to infection by PLRV by direct inoculation, plant-to-plant spread, or natural exposure. In field experiments, plants were also naturally exposed to the complex of viruses that occur in the region. Both the greenhouse and field tests failed to show any change in the type or severity of disease symptoms, and all viruses isolated were previously known to infect the plants and had the expected transmission characteristics (Ref. 78). These results suggest that viral interactions leading to evolution of new viruses and/or more severe viral disease are rare events.

A two-year experiment with transgenic melon and squash expressing coat protein genes of an aphid-transmissible strain of CMV failed to find evidence that either recombination or heterologous encapsidation enabled spread of an aphid non-transmissible strain of CMV in the field (Ref. 79). A similar experiment used transgenic squash expressing coat protein genes of an aphid-transmissible strain of watermelon mosaic virus (WMV). Plants were mechanically inoculated with an aphid non-transmissible strain of ZYMV, and subsequent transmissions of the virus (assumed to be vectored by aphids) were assessed. Infections of ZYMV were not detected in nontransgenic fields, but the virus infected up to 2% of plants in transgenic fields. Several lines of evidence suggested ZYMV infection was mediated by the WMV PVC-protein heterologously encapsidating the ZYMV viral genome. However, the virus spread over short distances, and transmission at a low rate failed to lead to an epidemic of ZYMV in fields of WMV-resistant transgenic squash despite the presence of optimal conditions for transmission (Ref. 67). These results support the contention that even if heterologous encapsidation involving a PVC-protein were to occur, the impact is likely to be limited because each plant infection requires a rare event to occur. Natural processes of viral infection can be at least an order of magnitude more efficient and lead to relatively greater impacts (Ref. 67).

An experiment to assess the biological and genetic diversity of California CMV isolates sampled before and after deployment of transgenic melon containing the CMV coat protein gene documented only one CMV isolate that had significant sequence changes after infecting the transgenic squash. However, this isolate did not result from recombination; most likely it reflected a random colonization on new host plants from a mixed population inoculum (Ref. 80). The only field experiment to directly assess the effect of recombination in a transgenic plant containing a PVCP-PIP found no detectable grapevine fanleaf virus (GFLV) recombinants containing the inserted coat protein sequence over the course of a four-year study (Ref. 81). Test plants consisted of nontransgenic scions grafted onto transgenic and nontransgenic rootstocks that were exposed over 3 years to GFLV infection at two sites. Analysis of challenging GFLV isolates revealed no difference in the molecular variability among isolates from 190 transgenic and 157 nontransgenic plants, or from plants within (253 individuals) or outside (94 individuals) of the two test sites.

5. Conclusions regarding viral interactions

The information in Units II.B.2-4 suggests that heterologous encapsidation very rarely leads to changes in virus epidemiology that could have any large-scale impact and that synergy in plants containing PVCP-PIPs also is unlikely to cause any widespread environmental harm. Consistent with these observations, the 2004 SAP that "except perhaps for a very few cases, neither heterologous encapsidation nor synergy should be considered to be of serious concern" (Ref. 31). The Agency believes that even in the very few cases mentioned by the SAP, concerns associated with these types of viral interactions are likely to be limited in scope (for reasons discussed in Units II.B.2-3) such that the determination can be made that they pose low risk to human health and the environment. EPA therefore concludes that PVCP-PIPs present low risk with respect to heterologous encapsidation and synergy and that PVCP-PIPs could be exempted without further qualification/requirements to address these endpoints.

However, EPA is not able to draw the same conclusions regarding recombination. Based on the available evidence (discussed in Unit II.B.1), EPA agrees with the conclusions of the 2004 SAP that "[i]n contrast to heterologous encapsidation and synergy, at least in theory, the impact of recombination could be much greater, since there is now abundant bioinformatic evidence that recombination has indeed, as long suspected, played a key role in the emergence of new viruses over evolutionary time" (Ref. 22).

The few field evaluations conducted (discussed in Unit II.B.4) suggest that adverse environmental effects due to recombination in transgenic plants containing PVCP-PIPs are unlikely to occur at least on a small scale over a short time period. However, large acreages of VCP-transgenic plants grown over many years may provide increased opportunity for rare events to occur that are unlikely to be detected in experimental studies (Ref. 75). In addition, none of the experimental systems described above would be predicted to involve viruses that would otherwise not be expected to interact in a mixed infection found in nature. Given the limited amount of field data available, particularly data relevant to the circumstances EPA has identified as being of highest concern (i.e., those that could lead to novel interactions), EPA would limit an exemption to those PVCP-PIPs for which novel viral interactions are unlikely to occur. When

EPA consulted the 2004 SAP about situations in which novel viral interactions might be a concern, the Panel agreed “that recombination is a concern when the two contributing viruses have not previously had a chance to recombine” (Ref. 22).

In addition to considering the potential for novel viral interactions to occur, EPA also considered whether transgenic plants containing PVCP-PIPs might present opportunity for a generally increased frequency of viral interactions given that a transgene expressed with a constitutive promoter could be present in all cells of the plant at all times. In natural mixed infections viruses must simultaneously replicate in the same cellular compartment for their RNA to be able to interact. However, when a virus invades a cell, it often replicates and then moves to other cells within the plant. The RNA remaining in the initially infected cell becomes encapsidated and may no longer be available for interactions with another invading virus (Ref. 82). When EPA presented this issue to the 2004 SAP, the panel responded that “no increase in heterologous encapsidation should be anticipated in PVCP-PIP plants” and “the important questions are not the relative likelihood for recombination to occur, but rather whether recombinants in transgenic plants are different from those in non-transgenic plants and whether they are viable” (Ref. 22). Thus, EPA’s current approach focuses on situations in which novel recombination events could occur due to the presence of a PVCP-PIP.

6. Categorical exemption criterion

In developing the categorical exemption for a subset of PVCP-PIPs in which a developer could self-determine whether the criteria were met, EPA seeks to identify those situations that clearly pose low risk with respect to viral interactions, i.e., those situations in which recombination in a transgenic plant would involve segments of viruses that already have the opportunity to recombine in a natural, mixed infection.

A PVCP-PIP would meet criterion (b) under paragraph (1) if the viral pathotype used to create the PVCP-PIP has naturally infected plants in the United States, its possessions, or territories and naturally infects plants of the same species as that containing the PVCP-PIP. The developer may make this determination. If the viral pathotype was isolated from a plant in the United States, its possessions, or territories that is of the same species as the plant containing the PVCP-PIP and was not subsequently modified, the PVCP-PIP meets this criterion. No further data or information would be needed to evaluate risks associated with recombination when paragraph (1) of criterion (b) is satisfied, and therefore no Agency review would be necessary.

The Agency asked the FIFRA SAP during the October 2004 meeting to what extent PVCP-PIPs in plants might present a potential concern should interactions with infecting viruses occur. The Panel expressed concern only “about certain limited situations” and stated that "in most cases there is little *a priori* reason to believe that recombinants between viruses and transgenes will be more of a problem than recombinants between two viruses infecting the same plant, unless transgenes are derived from severe or exotic isolates. The general recommendation to use mild, endemic isolates as the source of the transgene (e.g. Hammond et al. 1999) should minimize any potential for creation of novel isolates that would not equally easily arise in natural mixed infections" (Ref. 27).

The Agency's approach in paragraph (b)(1) is consistent with the SAP's recommendations because it excludes use of exotic virus isolates as the source of the PVCP-PIP transgene. When severe isolates are used, the PVCP-PIP may only meet paragraph (b)(1) if they were present in the natural system and therefore should pose no novel interactions. Paragraph (b)(1) is also intended to exclude from exemption PVCP-PIPs that are inserted into a plant species that is not naturally infected by the virus used to create the PVCP-PIP. Most PVCP-PIPs are created from viruses that do naturally infect the plant species into which they are inserted because greater efficacy is achieved when a virus most similar to the target virus is used to isolate the sequence used in the PVCP-PIP. However, virus-resistant transgenic plants have been created where this is not the case (Ref. 83). In these situations, a virus is introduced into a system where it does not naturally occur, and viruses with which it does not otherwise interact may be present in that system. The Agency cannot *a priori* determine that such interactions are safe because there is no experience upon which to base such a finding.

EPA means by the term "naturally infect" to infect by transmission to a plant through direct plant-to-plant contact (e.g., pollen or seed), an inanimate object (e.g., farm machinery), or vector (e.g., arthropod, nematode, or fungus). It does not include infection by transmission that occurs only through intentional human intervention. The Agency wants specifically to exclude transmission that occurs only through intentional human intervention, e.g., manual infection in a laboratory or greenhouse setting, because such transmission would have little relevance to normal human dietary exposure. EPA intends to include viruses that are likely to have been part of the human diet due to their ability to spread without intentional human intervention. EPA recognizes that humans may play an inadvertent role in infection (e.g., by transmitting the virus on farm machinery). Such unintentional (and often unavoidable) transmission can be an important means of virus transmission, and this mode of transmission would be included under "naturally infects."

EPA uses the term "viral pathotype" rather than the more generic term "virus" in response to the FIFRA SAP comment in October 2004 that "[n]ot all isolates of a virus infect and cause disease in all plant genotypes and, as a consequence, the unqualified use of the term "virus" when setting a condition for applicants to the Agency [is] not adequate in this context. It is therefore appropriate in the context of biosafety as well as virus epidemiology to recognize the value of defining specific viral pathotypes or host range variants."

7. Exemption criterion conditional on Agency determination

The Agency recognizes that many PVCP-PIPs may pose low risk with respect to recombination even though they fail to satisfy paragraph (1) of criterion (b). Therefore, EPA is considering an approach under which PVCP-PIPs that fail to meet paragraph (b)(1) could still meet criterion (b), subject to an Agency review to determine whether they meet a different set of conditions related to this issue. Under this approach, a PVCP-PIP would meet criterion (b) under paragraph (2) if the Agency determines either that (i) the properties of the viral pathotype that are determined by the coat protein gene used to create the PVCP-PIP are substantially similar to the properties of a viral pathotype that naturally infects plants in the United States, its possessions, or territories, and the viral pathotype used to create the PVCP-PIP naturally infects plants of the same species

as that containing the PVCP-PIP, or (ii) viruses that naturally infect the plant containing the PVCP-PIP are unlikely to acquire the coat protein sequence through recombination and produce a viable virus with significantly different properties than either parent virus.

With an Agency determination under paragraph (2) of criterion (b), EPA would create a criterion that would encompass a larger set of those PVCP-PIPs that pose low risk with respect to viral interactions than are covered under paragraph (1). However, even with an Agency determination, EPA must still define a criterion with sufficient precision such that the public would be able to understand what products would qualify and evaluate whether they would meet the standard for a FIFRA exemption. EPA seeks to define a characteristic of a PVCP-PIP that would indicate the viral interactions that could occur would be no different than would occur in a natural, mixed infection found in nature. PVCP-PIPs meeting the condition in paragraph (b)(2)(i) would pose low probability of risk with respect to viral interactions because the viral interactions that EPA determines could occur in that plant would not be substantively different than what could occur in a natural mixed infection in the United States involving that virus.

EPA believes that an Agency review would be needed to make this determination because it is more complicated than the relatively straightforward determination of whether paragraph (b)(1) is met based on knowledge of the plant from which the viral pathotype used to create the PVCP-PIP was isolated. If the viral pathotype was isolated from outside the United States, its possessions, or territories or the coat protein sequence was modified after isolation, a determination of substantial similarity would be based on consideration of similarity in the coat protein gene sequence of the pathotype used and of pathotypes found in the United States, its possessions, or territories. If information is available, EPA would evaluate the extent to which any significant sequence differences are likely to influence phenotypic properties of the virus. EPA's review would consider data from a number of different sources including virus coat protein sequence data from public repositories and developer-generated data on the natural range of variation of coat protein genes for particular viral pathotypes.

EPA believes that the risks associated with recombination arise when the potential recombinants would be unlike those expected in a natural mixed infection found in nature. The conditions in paragraphs (2)(a) and (2)(b)(i) address these concerns by ensuring that no novel viral interactions occur. Under paragraph (2)(b)(ii), a PVCP-PIP could qualify for exemption even when novel viral interactions could occur, providing steps were taken to significantly reduce the likelihood that an infecting virus would not acquire a portion of the PVCP-PIP coat protein sequence through recombination and produce a viable virus with significantly different properties than either parent virus. For example, the following methods for reducing the frequency of recombination might be relevant in evaluating a PVCP-PIP under paragraph 2(b)(ii): if the PVCP-PIP confers virus resistance through post-transcriptional gene silencing thereby greatly reducing the amount of RNA available for recombination; if the PVCP-PIP construct is designed to reduce the frequency of recombination (e.g., Refs. 84, 85, 86, 87, 88); or if the inserted coat protein sequence is only a relatively small portion of the naturally occurring sequence suggesting that viruses acquiring the region are unlikely to acquire a novel phenotype. EPA recognizes the comments of the 2004 SAP that "methods for minimizing recombination are only partially effective. For this reason, the question remains whether novel recombinants would be created in transgenic plants, and simply reducing the frequency of these events is not an answer to the question" (Ref. 31). However, a combination of two or more methods, or even perhaps a single

method in some cases, could be employed to reduce the expected frequency of recombination to a level that would support a determination that a PVCP-PIP would pose low risk with respect to viral interactions. Given that there is no universally applicable method for reducing recombination frequency, EPA believes an Agency review is needed to make this determination.

8. Other approaches

EPA's proposed exemption in 1994 did not contain any criteria related to viral interactions. However, since that time, many additional scientific papers and reviews have been published on this topic. Most affirm the general safety of PVCP-PIPs with respect to viral interactions, but some call into question assumptions of how generically this conclusion holds across all PVCP-PIPs. For example, although the 2000 NRC report stated that, "[m]ost virus-derived resistance genes are unlikely to present unusual or unmanageable problems that differ from those associated with traditional breeding for virus resistance," the NRC's report also suggested that their conclusions were based on the assumption that certain risk management strategies should or would be implemented, e.g., elimination of specific sequences to limit the potential for recombination (Ref. 26). EPA believes the Agency's 1994 conclusion of low probability of risk still holds for most PVCP-PIPs, but in order to grant an exemption under FIFRA, EPA must be able to make such a finding for all PVCP-PIPs covered by the exemption and must make its safety determination in the absence of any regulatory oversight under FIFRA that could ensure mitigation measures, such as those discussed in the NRC report, were employed. Therefore, it appears prudent at this time to limit any exemption with a criterion that restricts the potential for novel recombination events, as these have been identified as the rare situation in which viral interactions in plants containing a PVCP-PIP may lead to adverse environmental effects.

EPA presented a set of conditions to the 2004 SAP and asked whether they would significantly reduce either the novelty or frequency of viral interactions in plants containing PVCP-PIPs such that the Agency would not need to regulate the PVCP-PIP (Ref. 22). The first condition was that "the genetic material of the PVCP-PIP is translated and/or transcribed in the same cells, tissues, and developmental stages naturally infected by every virus from which any segment of a coat protein gene used in the PVCP-PIP was derived." EPA considered such a condition because with a PVCP-PIP, plants may express viral genes in cells and/or tissues that the virus does not normally infect. Genetic promoters currently used in most transgenic plants cause constitutive expression of transgenes at developmental stages that might otherwise be unaffected by viral infection and often in tissues that the virus does not normally infect (Ref. 82). For example, luteoviruses are normally expressed only in phloem tissue, but the cauliflower mosaic virus (CaMV) promoter drives expression of luteoviral coat protein in all plant cells. Some evidence suggests that in natural infections different viruses have different temporal or spatial expression patterns that would limit their interactions (Refs. 34, 89, 90). However, the 2004 SAP concluded that such a condition would be of limited utility because "[m]ost plant viruses are present in a wide range of cell and tissue types" (Ref. 22).

The second condition presented to the 2004 SAP was that "the genetic material of the PVCP-PIP contains coat protein genes or segments of coat protein genes from viruses established throughout the regions where the crop is planted in the United States and that naturally infect the

crop into which the genes have been inserted." EPA considered the first part of this criterion because plants may be engineered with PVCP genes from an exotic strain of a virus that may be more virulent or have other properties different from endemic isolates. Interactions with such virus sequences could potentially change the epidemiology or pathogenicity of viruses infecting plants containing these sequences. The 2004 SAP concurred that "using such an exotic PVCP gene would open possibilities for novel interactions." EPA's current criterion (b) thus would exclude exotic coat protein genes from exemption unless steps have been taken to reduce the frequency of recombination. EPA considered the second part of this criterion because in heterologous resistance, a plant may be resistant to infection by a particular virus in spite of having the coat protein gene of another virus incorporated into its genome. For example, PVCP genes from LMV were used to provide resistance to PVY in tobacco which is not infected by LMV (Ref. 91). In such plants, LMV might have a new opportunity to interact with viruses that infect tobacco. The 2004 Panel concluded that "[w]hat is described here is most often implemented: in designing a PVCP transgene, better efficacy is often observed if it is similar as possible to the target virus." Nevertheless, EPA believes that such a condition is appropriate given that PVCP-PIPs may be developed using heterologous resistance. EPA's current approach thus excludes from exemption PVCP-PIPs used in plants that the virus used to create the PVCP-PIP does not naturally infect unless steps have been taken to reduce the frequency of recombination.

The third condition presented to the 2004 SAP was that "the PVCP-PIP has been modified by a method scientifically documented to minimize recombination (e.g., deletion of the 3' untranslated region of the coat protein gene). As discussed above, EPA recognizes the comments of the 2004 SAP that "methods for minimizing recombination are only partially effective. For this reason, the question remains whether novel recombinants would be created in transgenic plants, and simply reducing the frequency of these events is not an answer to the question" (Ref. 31). However, EPA believes that a combination of two or more methods, or even perhaps a single method in some cases, could be employed such that the expected frequency of recombination would be reduced to a level that would support determination that a PVCP-PIP would pose low risk with respect to viral interactions. EPA intends that paragraph (b)(2)(ii) would allow the Agency to make this determination after review.

The fourth condition presented to the 2004 SAP was that "the PVCP-PIP has been modified by a method scientifically documented to minimize heterologous encapsidation or vector transmission, or there is minimal potential for heterologous encapsidation because no protein from the introduced PVCP-PIP is produced in the transgenic plant or the virus does not participate in heterologous encapsidation in nature." The 2004 SAP concluded that "[t]his method can … be considered seriously if deemed necessary." However, the Agency concluded (as discussed above in Unit II.B.2) that such methods are not necessary because heterologous encapsidation is so rarely likely to be of any significant ecological concern.

C. Production of proteins.

PVCP-PIPs contain plant virus coat protein sequences that may lead to protein production in the plant in which the sequences are inserted. EPA thus must consider the safety of any potentially expressed proteins when proposing criteria to evaluate PVCP-PIPs for possible exemption.

EPA has to consider human dietary, nontarget, and occupational exposure risks in evaluating the safety of PVC-proteins. Readers are referred to EPA's assessment of human dietary exposure risks as well as other non-occupational exposure in Attachment II: Draft Approach to Exempting Certain PVC-Proteins from the Requirement of a Tolerance under FFDCA. Many, if not all, of the considerations used to evaluate the potential for novel exposures in nontargets can be directly extrapolated from the discussion on the history of safe exposure to naturally occurring plant virus coat proteins found in Attachment II.

EPA consulted the 2004 SAP about possible nontarget effects of PVC-proteins. The panel confirmed that PVC-proteins within the range of natural variation of the virus would not be anticipated to present risks to nontarget organisms, concluding that, "[l]ethal effects in animal life after feeding on PVCP-PIP plants are highly unlikely because plant viruses are not known to have deleterious effects on animal life. Additionally, animals routinely feed on non-engineered virus-infected plants and do not die…. [S]ublethal effects are not expected to be manifested in animal life, again because wildlife and insects regularly feed on non-engineered virus-infected plants with no apparent sublethal damage" (Ref. 31).

1. Categorical exemption criterion

In developing the categorical exemption for a subset of PVCP-PIPs in which a developer could self-determine whether the criteria were met, EPA seeks to identify those situations that clearly pose low risk with respect to protein production because the proteins produced would be within the range of natural variation. EPA wants to ensure that a long history of safe human and nontarget exposure has occurred for any PVC-protein produced from a PVCP-PIP that would qualify for an exemption. A PVCP-PIP would meet criterion (c) under paragraph (1) if a product developer self-determines that the genetic material encodes only a single contiguous portion of each unmodified viral coat protein. This would include multiple proteins expressed from a single PVCP-PIP construct, but not chimeric proteins.

The requirement that the genetic material encode "only a single contiguous portion of each unmodified viral coat protein," would exclude residues of modified PVC-proteins. For example, PVC-proteins containing insertions, internal deletions, or amino acid substitutions would be excluded, as would be chimeric proteins that are encoded by a sequence constructed from portions of two or more different plant virus coat protein genes. EPA is considering whether to exclude such PVC-proteins from the self-determining part of the exemption in response to the advice of the FIFRA SAP in October 2004 that, "[t]here was general agreement that an allergenicity assessment would be appropriate for insertions or deletions, except perhaps for terminal deletions that do not affect overall protein structure." However, insufficient information exists at this time to allow EPA to describe *a priori* a criterion that would ensure all PVC-proteins with such modifications fall within the base of experience supporting an exemption. At this point in time, it is not possible to make a categorical risk assessment finding that insertions

or internal deletions are unlikely to change the characteristics of any protein produced. Thus, EPA would follow the prudent course for paragraph (1) of criterion (c) and allow neither modification.

EPA believes the phrase “a single contiguous portion” conveys the concept that segments of PVC-proteins that are identical to an unmodified coat protein would also be exempt. EPA believes the exemption of segments is supported by the experience base EPA is relying on to develop an exemption because it is probable that segments of coat proteins exist in nature due to processes such as incomplete translation of transcripts and partial degradation of proteins. Incomplete translation may occur due to routine replication errors causing a ribosome to dissociate from an RNA transcript or if mutation introduces a premature stop codon, i.e., a nonsense mutation. Truncated plant virus coat proteins are indeed known to occur in nature (Ref. 92). Thus, PVC-proteins that are truncated forms of naturally occurring plant virus coat proteins would not significantly increase the likelihood of exposure to a toxic or allergenic protein since humans are currently exposed to them in the diet along with complete plant virus coat proteins.

The Agency is considering whether also to include in the categorical exemption, i.e., without Agency review, amino acid sequences containing terminal deletion(s) and/or an additional N-terminal methionine residue. The AUG codon for methionine initiates translation in eukaryotes (Ref. 93). Among certain viruses such as the Potyviridae, the coat protein is produced as part of a polyprotein, so the coding region for the coat protein is excised from the genetic material encoding the polyprotein to create a PVCP-PIP and thus normally lacks a start codon. Insertion of an AUG codon allows for PVC-protein expression, which may be needed to confer virus resistance. EPA believes the addition of a single, N-terminal methionine residue would be unlikely to affect a PVC-protein’s toxicity or allergenicity relative to a naturally occurring plant virus coat protein.

If the genetic material encodes only a single contiguous portion of an unmodified viral coat protein, no novel exposures to humans or nontarget organisms are likely to occur because these PVC-proteins are identical to plant viral coat proteins that are widespread in the plant kingdom, as most plants are infected by at least one virus. EPA is relying on this history of safe exposure when considering whether to exempt certain PVCP-PIPs from regulation under FIFRA. The Agency believes that when such a PVCP-PIP is used, the PVCP-PIP would pose low probability of risk with respect to protein production. EPA believes that no further data or information would be needed to evaluate this issue when paragraph (1) of criterion (c) is satisfied, and therefore no Agency review would be necessary.

2. Exemption criterion conditional on Agency determination

The Agency acknowledges that many PVCP-PIPs may pose low risk with respect to concerns associated with protein production even though they fail to satisfy paragraph (1) of criterion (c). EPA would review such PVCP-PIPs that fail to meet paragraph (c)(1) under slightly different factors that the Agency believes also ensure that qualifying PVCP-PIPs pose low risk with respect to concerns associated with protein production. Therefore, a PVCP-PIP would also meet criterion (c) under paragraph (2) if the Agency determines that the genetic material (i) encodes a

protein that is minimally modified from a coat protein from a virus that naturally infects plants, or (ii) produces no protein.

The Agency's rationale for concluding no novel exposures to proteins are associated with PVCP-PIPs would cover only those PVC-proteins that are not significantly different from naturally occurring plant viral coat proteins. For PVCP-PIPs that contain modified genetic material encoding a PVC-protein that is not identical or not minimally modified from a naturally occurring plant virus coat protein, the base of experience upon which EPA relies to support exempting such proteins would not apply. Therefore, were such a PVC-protein to be produced from the PVCP-PIP, EPA would not be able to make the determination that the PVCP-PIP poses a low probability of risk to humans and the environment and will not generally cause unreasonable adverse effects on the environment even in the absence of regulatory oversight under FIFRA. For discussion of the concept of "minimally modified" see Unit II.D.2 of Attachment II: Draft Approach to Exempting Certain PVC-Proteins from the Requirement of a Tolerance under FFDCA.

EPA developed paragraph (2) of criterion (c) because the Agency recognizes that PVCP-PIP developers may wish to modify PVCP-PIP constructs to achieve certain product development goals such as greater efficacy, and such modifications might result in changes to the protein(s) produced. Many modifications to the genetic material may be so minor that they are unlikely to cause changes to the protein that would be significant from a human or nontarget organism perspective. Under paragraph (c)(2) EPA may consider such insertions or internal deletions on a case-by-case basis. Many of the modifications are likely to produce proteins that fall within the range of natural variation of the virus. However, it is not currently possible clearly to define the range of variation of viruses in general or even of any particular virus as discussed in Unit II.C of Attachment II. Therefore, paragraph (2)(i) of criterion (c) requires an Agency review to determine qualification.

PVCP-PIPs are known to have at least two mechanisms to confer virus resistance. Resistance may be either protein-mediated, in which the level of resistance is correlated with the level of protein expression, or it may be RNA-mediated, in which the level of resistance is not correlated with the level of protein expression. In the case of RNA-mediated resistance, little to no PVC-protein may be produced from the PVCP-PIP. In such cases, little to no risk due to protein production would be associated with the PVCP-PIP. However, the Agency believes that it would not be possible at this time to describe *a priori* conditions that must be satisfied to ensure that no protein is produced by the PVCP-PIP. Therefore, paragraph (2)(ii) of criterion (c) requires an Agency review to determine qualification.

3. Other approaches

The approach EPA is currently considering is consistent with what EPA has always intended. EPA has never intended that any proposed exemption for PVCP-PIPs would cover those that produce proteins significantly different from those that occur naturally (November 23, 1994, 59 FR at 60539; see in particular July 19, 2001, 66 FR 37865). EPA's approach discussed here relies on the known history of safe exposure to coat proteins of naturally occurring plant viruses.

However, this rationale would only cover those PVC-proteins that are not significantly different from naturally occurring plant viral coat proteins. For modified PVCP-PIPs, the base of experience upon which EPA relies for support of an exemption might not be relevant. In some cases, EPA might not be able to make the determination that the PVCP-PIP poses a low probability of risk to humans and the environment and will not generally cause unreasonable adverse effects on the environment even in the absence of regulatory oversight under FIFRA.

D. Other Definitions

Under this exemption approach, a plant-incorporated protectant based on a plant virus coat protein gene (PVCP-PIP) would be defined to mean a plant-incorporated protectant based on one or more genes that encode a coat protein of a virus that naturally infects plants. This includes PVCP-PIPs that produce no protein. A PVCP-PIP may contain multiple plant virus coat protein genes, or segments thereof, translated as individual proteins. A PVCP-PIP may also contain multiple plant virus coat protein genes, or segments thereof, translated as a single, chimeric protein. In this context, the word “segment” has the commonly accepted meaning (Ref. 94), i.e., a “part cut off from the other parts” of the whole coat protein.

The definition of a PVCP-PIP would contain the phrase “naturally infects plants.” Including this phrase in the definition would specifically limit an exemption by requiring that the virus coat protein gene upon which the PVCP-PIP is based come exclusively from a plant virus. This limitation is intended to exclude from the definition any coat proteins of plant viruses that have been modified with sequences from animal or human viruses. EPA includes this concept in response to comment received from the public on earlier documents pertaining to PVCP-PIPs.

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Appendix: Index of Exemption Criteria

(a) Criterion a is satisfied if either paragraph 1 or paragraph 2 applies:

(1) The plant containing the PVCP-PIP is one of the following: almond (*Prunus communis*), apricot (*Prunus armeniaca*), asparagus (*Asparagus officinale*) avocado (*Persea americana*), banana (*Musa acuminata*), barley (*Hordeum vulgare*), bean (*Phaseolus vulgaris*), black-eyed pea (*Vigna unguiculata*), cacao (*Theobroma cacao*), celery (*Apium graveolens*), chickpea (*Cicer arietinum*), citrus (*Citrus* spp.), coffee (*Coffea arabicua*), corn (*Zea maize*), cucumber (*Cucumis sativus*), eggplant (*Solanum melongena*), guava (*Psidium guajava*), kiwi (*Actinidia* spp.), mango (*Mangifera indica*), nectarine (*Prunus persica*), okra (*Abelmoschus esculentus*), olive (*Olea europaea*), papaya (*Carica papaya*), parsley (*Petroselinum crispum*), pea (*Pisum sativum*), peach (*Prunus persica*), peanut (*Arachis hypogaea*), pineapple (*Ananas comosus*), pistachio (*Pistacia vera*), plum (*Prunus domestica*), potato (*Solanum tuberosum*), soybean (*Glycine max*), spinach (*Spinacia oleracea*), starfruit (*Averrhoa carambola*), taro (*Colocasia esculenta*), tomato (*Lycopersicon lycopersicum*), or watermelon (*Citrullus lanatus*).

(2) The Agency determines after review that the plant containing the PVCP-PIP

(i) is itself not a weedy or invasive species outside of agricultural fields in the United States, its possessions, or territories, and

(ii) does not have relatives outside of agricultural fields in the United States, its possessions, or territories that are weedy or invasive species or endangered/threatened species with which it can produce viable hybrids in nature.

(b) Criterion b is satisfied if either paragraph 1 or paragraph 2 applies:

(1) The viral pathotype used to create the PVCP-PIP has naturally infected plants in the United States, its possessions, or territories and naturally infects plants of the same species as that containing the PVCP-PIP.

(2) The Agency determines after review that

(i) the properties of the viral pathotype that are determined by the coat protein gene used to create the PVCP-PIP are substantially similar to the properties of a viral pathotype that naturally infects plants in the United States, its possessions, or territories, and the viral pathotype used to create the PVCP-PIP naturally infects plants of the same species as that containing the PVCP-PIP, or

(ii) viruses that naturally infect the plant containing the PVCP-PIP are unlikely to acquire the coat protein sequence through recombination and produce a viable virus with significantly different properties than either parent virus.

(c) Criterion c is satisfied if either paragraph 1 or paragraph 2 applies:

(1) The genetic material encodes only a single contiguous portion of each unmodified viral coat protein. This would allow multiple PVC-proteins that could each separately qualify for the exemption. Chimeric PVC-proteins would not qualify.

(2) The Agency determines after review that the genetic material

(i) encodes a protein that is minimally modified from a coat protein from a virus that naturally infects plants, or

(ii) produces no protein.